

FORM PTO-1390 (Modified)  
(REV 11-98)

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER

TRANSMITTAL LETTER TO THE UNITED STATES  
DESIGNATED/ELECTED OFFICE (DO/EO/US)  
CONCERNING A FILING UNDER 35 U.S.C. 371

33207-PCT USA

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR

09/554024

INTERNATIONAL APPLICATION NO.  
PCT/FR98/02375INTERNATIONAL FILING DATE  
6 November 1998PRIORITY DATE CLAIMED  
7 November 1997

## TITLE OF INVENTION

GENE CODING FOR THANATIN, VECTOR CONTAINING SAME AND RESULTING TRANSFORMED  
DISEASE-RESISTANT PLANTS

## APPLICANT(S) FOR DO/EO/US

FREYSSINET, Georges; DEROSE, Richard; and HOFFMANN, Jules

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
  - a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
  - b. ☒ has been transmitted by the International Bureau.
  - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ A copy of the International Search Report (PCT/ISA/210).
8. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
  - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
  - b. ☐ have been transmitted by the International Bureau.
  - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
  - d. ☒ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
11. ☐ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

## Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☒ Certificate of Mailing by Express Mail
20. ☒ Other items or information:

Published WO 99/24594, Preliminary Amendment, a postcard, and a check in the amount of \$1,020.

Express Mail No. EJ594514924US

Date of Deposit: May 8, 2000

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR <b>09/554024</b>	INTERNATIONAL APPLICATION NO. <b>PCT/FR98/02375</b>	ATTORNEY'S DOCKET NUMBER <b>33207-PCT USA</b>
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21. The following fees are submitted.:

**BASIC NATIONAL FEE ( 37 CFR 1.492 (a) (1) - (5)) :**

- ☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO ..... **\$970.00**
- ☒ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO ..... **\$840.00**
- ☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO ..... **\$690.00**
- ☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) ..... **\$670.00**
- ☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) ..... **\$96.00**

**ENTER APPROPRIATE BASIC FEE AMOUNT =****CALCULATIONS PTO USE ONLY****\$840.00**

Surcharge of **\$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).

**\$0.00**

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE
Total claims	30 - 20 =	10	x \$18.00
Independent claims	2 - 3 =	0	x \$78.00

**\$180.00****\$0.00**Multiple Dependent Claims (check if applicable). ☐**\$0.00****TOTAL OF ABOVE CALCULATIONS =****\$1,020.00**

Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) (check if applicable). ☐

**\$0.00****SUBTOTAL =****\$1,020.00**

Processing fee of **\$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)).

**\$0.00****TOTAL NATIONAL FEE =****\$1,020.00**

Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) (check if applicable). ☐

**\$0.00****TOTAL FEES ENCLOSED =****\$1,020.00**

Amount to be:

refunded

\$

charged

\$

☒ A check in the amount of **\$1,020.00** to cover the above fees is enclosed.

☐ Please charge my Deposit Account No. \_\_\_\_\_ in the amount of \_\_\_\_\_ to cover the above fees.  
A duplicate copy of this sheet is enclosed.

☒ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. **02-4377** A duplicate copy of this sheet is enclosed.

**NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.**

SEND ALL CORRESPONDENCE TO:

**Janet M. MacLeod**  
**Baker Botts LLP**  
**30 Rockefeller Plaza**  
**New York, NY 10112-028**

SIGNATURE

**Janet M. MacLeod**

NAME

**35,263**

REGISTRATION NUMBER

**8 May 2000**

DATE

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicants : FREYSSINET, Georges et al.  
Serial No. : To be assigned  
Filed : 6 November 1998  
For : GENE CODING FOR THANATIN, VECTOR  
CONTAINING SAME AND RESULTING  
TRANSFORMED DISEASE-RESISTANT PLANTS

**Express Mail Mailing No. EJ594514924US**

**PRELIMINARY AMENDMENT**

Assistant Commissioner of Patent  
Box PCT  
Washington, D.C., 20231

Sir or Madam:

Prior to examination of the above-identified application, please make the following amendments:

**IN THE CLAIMS:**

Claim 5, Line 1: please delete "one of claims 1 to 4" and substitute therefor --claim 1--.

Claim 13, Line 4: please delete "claims 1 to 9" and substitute therefor --claim 1--.

Claim 15, Line 1: please delete "one of claims 13 or 14" and substitute therefor --claim 13--.

Claim 16, Line 3: please delete "claims 13 to 15" and substitute therefor --claim 13--.

Claim 19, Line 2: please delete "claims 13 to 15" and substitute therefor --claim 13--.


- Claim 23, Lines 2-3: please delete "claim 1 to 9 or a chimeric gene according to claims 13 to 15" and substitute therefor --claim 1--.
- Claim 26, Line 2: please delete "one of claims 24 or 25" and substitute therefor --claim 24--.
- Claim 27, Line 1: please delete "one of claims 24 to 26" and substitute therefor--claim 24--.
- Claim 28, Lines 3-4: please delete "claims 1 to 9 or a chimeric gene according to one of claims 13 to 15" and substitute therefor --claim 1--.
- Claim 29, Lines 3-4: please delete "claims 1 to 9 or a chimeric gene according to claims 13 to 15" and substitute therefor --claim 1--.
- Claim 30, Line 1: please delete "one of claims 28 or 29" and substitute therefor --claim 28--.
- Claim 30, Line 2: please delete "one of claims 16 to 18".

REMARKS

The forgoing amendments have been made to remove multiple dependencies.  
Favorable consideration and allowance of all pending claims is respectfully requested.

Respectfully submitted,  
BAKER BOTTS LLP

Dated: May 8, 2000

  
Janet M. MacLeod  
Reg. No. 35,263  
Attorney for the Applicant  
Tel. (212) 705-5000

414 Rec'd PCT/PTO 1 6 OCT 2000

FILE NO. A33207-PCT-USA-072667.0132

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : FREYSSINET, Georges et al.  
Serial No. : 09/554,024 Examiner:  
Filed : May 8, 2000 Group Art Unit:  
For : GENE CODING FOR THANATIN, VECTOR CONTAINING  
SAME AND RESULTING TRANSFORMED DISEASE-  
RESISTANT PLANTS

PRELIMINARY AMENDMENT

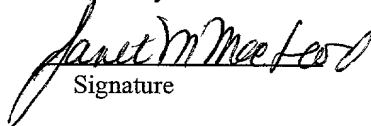
I hereby certify that this paper is being deposited with the United States  
Postal Service as first class mail in an envelope addressed to:  
Assistant Commissioner for Patents, Washington, D.C. 20231

October 11, 2000

Date of Deposit

Janet M. MacLeod

Attorney Name

  
Signature

35,263

PTO Registration No.

October 11, 2000

Date of Signature

Assistant Commissioner for Patents

Washington, D.C. 20231

**BOX PCT**

Sir:

Prior to examination of the above-identified application, please amend the  
application as follows:

IN THE SPECIFICATION:

Page 3, line 26, after "(I)" insert --(SEQ ID NO: 14)

Page 15, line 25, after "Oligo 1" insert --(SEQ ID NO: 6)--.

Page 16, line 1, after "Oligo 2" insert --(SEQ ID NO: 7)--.

Page 16, line 25, after "Oligo 3" insert --(SEQ ID NO: 8)--.

Page 17, line 1, after "Oligo 4" insert --(SEQ ID NO: 9)--.

Page 19, line 6, after "Oligo 5" insert --(SEQ ID NO: 10--).

Page 19, line 10, after "Oligo 6" insert --(SEQ ID NO: 11)--.

Page 21, line 13, after "Oligo 7" insert --(SEQ ID NO: 12)--.

Page 21, line 17, after "Oligo 8" insert --(SEQ ID NO:13)--.

After Page 29, delete the Sequence Listing and substitute therefor the attached Sequence Listing.

After Page 29, insert the attached separate page containing the Abstract.

**REMARKS**

The specification has been amended to include a substitute Sequence Listing and sequence identification numbers, and an abstract. No new matter has been added.

PATENT

The Sequence Listing is submitted herewith in paper and computer readable copies. The undersigned states that the contents of the paper and computer readable copies of the Sequence Listing are the same and contain no new matter.

In view of the foregoing comments and amendments, favorable consideration and allowance of all pending claims is respectfully requested.

Respectfully submitted,

  
Louis S. Sorell  
Patent Office Reg. No. 32,439

Janet M. MacLeod.  
Patent Office Reg. No. 35,263

Attorneys for Applicants  
(212) 408-2597

Enclosures

**ABSTRACT**

The invention concerns a nucleic acid sequence coding for thanatin, a vector containing it for transforming a host, and plants and plant cells transformed by the nucleic acid.



# SEQUENCE LISTING

<110> Freyssinet, Georges  
Deroose, Richard  
Hoffman, Jules

<120> Gene Coding for Thanatin, Vector  
Containing Same and Resulting Transformed Disease-Resistant  
Plants

<130> A33207-PCT-USA

<140> 09/554,024

<141> 2000-05-08

<150> PCT/FR98/02375

<151> 1998-11-06

<150> FR 97/14,263

<151> 1997-11-07

<160> 14

<170> FastSEQ for Windows Version 3.0

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<211> 33

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<213> Psodius maculiventis

<220>

<221> CDS

<222> (1)...(33)

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1 5 10

33

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<212> DNA

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<222> (1)...(63)

<223> Derived from Psodius maculiventis

<400> 2



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Met Gly Phe Val Leu Phe Ser Gln Leu Pro Ser Phe Leu  
1 5 10

ctt gtg tct act ctt ctt ctt ttc ctt gtg atc tct cac tct tgc cgt 98  
Leu Val Ser Thr Leu Leu Leu Phe Leu Val Ile Ser His Ser Cys Arg  
15 20 25

gcc ggt tcc aag aag cca gtg cca atc atc tac tgc aac agg agg act 146  
Ala Gly Ser Lys Lys Pro Val Pro Ile Ile Tyr Cys Asn Arg Arg Thr  
30 35 40 45

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tcc 197

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ctctttcttct tttcc 75

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aaagatggaa gc 72

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<210> 13

<211> 93

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<220>

<223> Synthetic oligonucleotide

<400> 13

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gcgtttaaac ttaattaagt gtggcctgac tgg	93

<210> 14

<211> 13

<212> PRT

<213> Artificial Sequence

<220>

<223> Derived from thanatin

<221> VARIANT

<222> (1)...(1)

<223> Variable residue

<221> VARIANT

<222> (13)...(13)

<223> Variable residue

<400> 14

Xaa	Ile	Ile	Tyr	Cys	Asn	Arg	Arg	Thr	Gly	Lys	Cys	Xaa
1				5					10			

09/554024

PCT/FR98/02375

WO 99/24594

1

Gene encoding thanatin, vector containing it and  
disease-resistant transformed plants obtained

The subject of the present invention is a DNA  
5 sequence encoding thanatin, a vector containing it for  
the transformation of a host organism, and the method  
of transforming the said organism.

The invention relates more particularly to  
the transformation of plant cells and plants, the  
10 thanatin produced by the transformed plants conferring  
on them resistance to diseases, in particular of fungal  
origin.

An increasing need already exists for making  
plants resistant against diseases, in particular fungal  
15 diseases, in order to reduce, or even eliminate, the  
need for treatment with antifungal protection products,  
with a view to protecting the environment. One means of  
increasing this resistance to diseases consists in  
transforming the plants so that they produce substances  
20 capable of providing their defence against these  
diseases.

Various substances of natural origin, in  
particular peptides, are known which exhibit  
bactericidal or fungicidal properties, in particular  
25 against the fungi responsible for plant diseases.  
However, the problem consists in finding such

substances which not only can be produced by transformed plants, but can still preserve their bactericidal or fungicidal properties and confer them on the said plants. For the purposes of the present invention, bactericidal or fungicidal is understood to mean the actual bactericidal or fungicidal properties and the bacteriostatic and fungistatic properties.

Thanatin is a peptide produced by bacterial induction on adult *Psodius sp*, preferably *maculiventris*. Its preparation by bacterial induction is described in patent application FR 2,733,237, as well as its antifungal and antibacterial properties *in vitro*.

After having first identified the thanatin gene, it was also found that it could be inserted into a host organism, in particular a plant, in order to express the thanatin and confer on the said host organism properties of resistance to fungal diseases and to diseases of bacterial origin, providing a particularly advantageous solution to the problem stated above.

The subject of the invention is therefore first a nucleic acid fragment encoding thanatin, a chimeric gene comprising the said fragment encoding thanatin as well as heterologous regulatory elements at the 5' and 3' positions which can function in a host organism, in particular in plants, and a vector for transforming the host organisms containing this

chimeric gene, and the transformed host organism. It also relates to a transformed plant cell containing at least one nucleic acid fragment encoding thanatin and a disease-resistant plant containing the said cell, in particular regenerated from this cell. It finally relates to a method of transforming plants to make them resistant to diseases, in which method a gene encoding thanatin is inserted by means of an appropriate vector.

Thanatin is understood to mean according to the invention any peptide comprising essentially the peptide sequence of 11 amino acids which is described in patent application FR 2,733,237, as well as the equivalent homologous sequences in which certain amino acids are replaced by different but equivalent amino acids at sites which do not induce substantial modification of the antifungal or antibacterial activity of the said homologous sequence. Peptide sequence comprising essentially the peptide sequence described in patent application FR 2,733,237 is understood to mean not only the sequence defined by the sequence identifier No. 1 (SEQ ID NO 1), but also such a sequence comprising at either of its ends, or at both, peptide residues necessary for its expression and targeting in a host organism, in particular a plant cell or a plant.

Thanatin is a peptide of formula (I):

Xaa-Ile Ile Tyr Cys Asn Arg Arg Thr Gly Lys Cys-Xab

(I)



in which:

Xaa is  $\text{NH}_2$  or a variable residue having a sequence comprising from 1 to 10 amino acids, and

Xab is OH or a variable residue having a  
5 sequence comprising from 0 to 5 amino acids.

Advantageously, when Xaa comprises at least one amino acid, the latter is one of the 20 base amino acids and more particularly chosen from the group comprising Gly, Ser, Lys, Pro and Val. When Xab  
10 comprises at least one amino acid, the latter is one of the 20 base amino acids and more particularly chosen from the group comprising Gln, Arg and Met.

According to a preferred embodiment of the invention, the two cysteine residues of the peptide of  
15 formula (I) form an intramolecular disulphide bridge.

The present invention therefore relates first to a nucleic acid, in particular a DNA, fragment encoding the thanatin defined above. It may be, according to the invention, a fragment isolated from  
20 *Psodius sp*, preferably *maculiventris*, or alternatively a derived fragment, suitable for the expression of thanatin in the host organism where the peptide will be expressed. The nucleic acid fragment may be obtained using standard methods of isolation and purification,  
25 or alternatively by synthesis according to the customary techniques of successive hybridizations of synthetic oligonucleotides. These techniques are in particular described by Ausubel et al.

According to the present invention, "nucleic acid fragment" is understood to mean a nucleotide sequence which may be of the DNA or RNA type, preferably of the DNA, in particular cDNA, especially double-stranded, type.

According to one embodiment of the invention, the nucleic acid fragment encoding thanatin comprises the DNA sequence described by the sequence identifier No. 1 (SEQ ID NO 1), a homologous sequence or a sequence complementary to the said sequence.

Advantageously, the nucleic acid fragment according to the invention comprises the DNA sequence described by the sequence identifier No. 2 (SEQ ID NO 2), a homologous sequence or a sequence complementary to the said sequence.

"Homologous" is understood to mean according to the invention a nucleic acid fragment having one or more sequence modifications relative to the nucleotide sequence described by the sequence identifier No. 1 or No. 2 and encoding thanatin. These modifications may be obtained according to the customary mutation techniques, or alternatively by choosing the synthetic oligonucleotides used in the preparation of the said sequence by hybridization. Given the multiple combinations of nucleic acids which may lead to the expression of the same amino acid, the differences between the reference sequence described by the sequence identifier No. 1 or No. 2 and the homologue

may be great, especially since a DNA fragment of less than 100 nucleic acids in size, which can be produced by synthesis, is involved. Advantageously, the degree of homology will be at least 70% relative to the  
5 reference sequence, preferably at least 80%, more preferably at least 90%. These modifications are generally neutral, that is to say that they do not affect the primary sequence of the resulting thanatin.

The present invention also relates to a  
10 chimeric gene (or an expression cassette) comprising a coding sequence as well as heterologous regulatory elements at the 5' and 3' positions which can function in a host organism, in particular plant cells or plants, the coding sequence comprising at least one DNA  
15 fragment encoding thanatin as defined above.

Host organism is understood to mean any higher or lower mono- or pluricellular organism into which the chimeric gene according to the invention may be introduced, for the production of thanatin. It  
20 consists of in particular bacteria, for example *E. coli*, yeasts, in particular of the genera *Saccharomyces* or *Kluyveromyces*, or preferably plant cells and plants.

"Plant cell" is understood to mean according  
25 to the invention any cell derived from a plant and which may constitute undifferentiated tissues such as calli, and differentiated tissues such as embryos, plant portions, plants or seeds.

"Plant" is understood to mean according to the invention any differentiated multicellular organism capable of photosynthesis, in particular monocotyledones or dicotyledones, more particularly  
5 cultivated plants intended or otherwise as animal feed or for human consumption, such as maize, wheat, colza, soya bean, rice, sugar cane, beet, tobacco, cotton and the like.

The regulatory elements necessary for the  
10 expression of the DNA fragment encoding thanatin are well known to persons skilled in the art depending on the host organism. They comprise in particular promoter sequences, transcription enhancers, transit peptides, terminator sequences, including start and stop codons.  
15 The means and methods for identifying and selecting the regulatory elements are well known to persons skilled in the art.

The nucleic acid fragment according to the invention may also comprise a nucleic acid sequence  
20 fused in 5' and/or 3' to the sequence encoding thanatin, so as to obtain a "protein-thanatin" fusion protein, whose cleavage by the enzymatic systems of the host organism allows the release of thanatin. This thanatin-fused protein may be a signal peptide or a transit  
25 peptide which makes it possible to control and orient the production of thanatin in a specific manner in a part of the host organism, such as for example the cytoplasm, the cell membrane, or in the case of plants

in a particular type of tissue or in the extracellular matrix.

According to one embodiment, the transit peptide may be a signal for chloroplast or  
 5 mitochondrial addressing, which transit peptide is then cleaved in the chloroplast or the mitochondria.

According to another embodiment of the invention, the signal peptide may be an N-terminal signal or "prepeptide", optionally in combination with  
 10 a signal responsible for retaining the protein in the endoplasmic reticulum, or a peptide for vacuolar addressing or "propeptide". The endoplasmic reticulum is the site where operations of maturation of the protein produced, such as for example the cleavage of  
 15 the signal peptide, are carried out by the "cellular machinery".

The invention relates more particularly to the transformation of plants. As regulatory promoter sequence in plants, there may be used any promoter  
 20 sequence of a gene which is expressed naturally in plants, in particular a promoter of bacterial, viral or plant origin such as, for example, that of a gene for the small subunit of ribulose biscarboxylase (RuBisCO) or of a plant virus gene, for example that of  
 25 cauliflower mosaic (CAMV 19S or 35S), or a promoter inducible by pathogens such as tobacco PR-1a or asparagus AoPRT-L, it being possible for any known suitable promoter to be used. Preferably, a regulatory

promoter sequence is used which promotes the overexpression of the coding sequence constitutively or inducibly by a pathogen attack, such as for example that comprising at least one histone promoter as  
5 described in application EP 0,507,698.

According to the invention, it is also possible to use, in combination with the regulatory promoter sequence, other regulatory sequences which are situated between the promoter and the coding sequence,  
10 such as transcription enhancers such as for example the tobacco mosaic virus (TMV) translation enhancer described in application WO 87/07644, or the tobacco etch virus (TEV) translation enhancer described by Carrington & Freed, or transit peptides, either single  
15 or double, and in this case optionally separated by an intermediate sequence, that is to say comprising, in the direction of transcription, a sequence encoding a transit peptide of a plant gene encoding a plastid localization enzyme, a portion of sequence of the N-  
20 terminal mature portion of a plant gene encoding a plastid localization enzyme, and then a sequence encoding a second transit peptide of a plant gene encoding a plastid localization enzyme consisting of a portion of sequence of the N-terminal mature portion of  
25 a plant gene encoding a plastid localization enzyme, as described in application EP 0,508,909. As transit peptide, there may be mentioned the signal peptide of the tobacco PR-1a gene described by Cornelissen et al.,

represented with its coding sequence by the sequence identifier No 3.

The sequence encoding the fusion protein signal peptide PR-1a-thanatın and this fusion protein  
5 also form part of the present invention. This sequence is in particular described by the sequence identifier No. 5, more particularly the coding part of this sequence, corresponding to bases 12 to 164.

As regulatory terminator or polyadenylation  
10 sequence, there may be used any corresponding sequence of bacterial origin, such as for example the nos terminator from *Agrobacterium tumefaciens*, or alternatively of plant origin, such as for example a histone terminator as described in application  
15 EP 0,633,317.

According to the present invention, the chimeric gene may also comprise a selectable marker suitable for the transformed host organism. Such selectable markers are well known to persons skilled in  
20 the art. They may be a gene for resistance to antibiotics, such as penicillin, or alternatively a gene for tolerance of herbicides for plants.

The present invention also relates to a cloning or expression vector for the transformation of  
25 a host organism containing at least one chimeric gene as defined above. This vector comprises, in addition to the above chimeric gene, at least one replication origin. This vector may consist of a plasmid, a cosmid,

a bacteriophage or a virus, transformed by the introduction of the chimeric gene according to the invention. Such transformation vectors, depending on the host organism to be transformed, are well known to  
5 persons skilled in the art and are widely described in the literature.

For the transformation of plant cells or plants, they may consist in particular of a virus which may be used for the transformation of developed plants  
10 and containing, in addition, its own elements for replication and expression. Preferably, the vector for transforming plant cells or plants according to the invention is a plasmid.

The subject of the invention is also a method  
15 of transforming host organisms, in particular plant cells, by integration of at least one nucleic acid fragment or a chimeric gene as defined above, which transformation may be obtained by any known appropriate means widely described in the specialist literature and  
20 in particular the references cited in the present application, more particularly by the vector according to the invention.

A series of methods consists in bombarding cells or protoplasts with particles to which the DNA  
25 sequences are attached. Another series of methods consists in using, as means of transferring into the plant, a chimeric gene inserted into a Ti plasmid from



*Agrobacterium tumefaciens* or an Ri plasmid from *Agrobacterium rhizogenes*.

Other methods may be used, such as microinjection or electroporation or alternatively  
5 direct precipitation by means of PEG.

Persons skilled in the art will make the choice of the appropriate method depending on the nature of the host organism, in particular the plant cell or the plant.

10 The subject of the present invention is also the host organisms, in particular plant cells or plants, transformed and containing an effective quantity of a chimeric gene comprising a sequence encoding the thanatin defined above.

15 The subject of the present invention is also the plants containing transformed cells, in particular the plants regenerated from transformed cells. The regeneration is obtained by any appropriate method which depends on the nature of the species, as for  
20 example described in the references above.

For the methods of transforming plant cells and of regenerating plants, the following patents and patent applications may be mentioned: US 4,459,355, US 4,536,475, US 5,464,763, US 5,177,010, US 5,187,073,  
25 EP 267,159, EP 604 662, EP 672 752, US 4,945,050, US 5,036,006, US 5,100,792, US 5,371,014, US 5,478,744, US 5,179,022, US 5,565,346, US 5,484,956, US 5,508,468, US 5,538,877, US 5,554,798, US 5,489,520, US 5,510,318,

US 5,204,253, US 5,405,765, EP 442,174, EP 486,233,  
EP 486,234, EP 539,563, EP 674,725, WO 91/02071 and  
WO 95/06128.

The present invention also relates to the  
5 transformed plants derived from the cultivation and/or  
crossing of the above regenerated plants, as well as  
the seeds of transformed plants.

The plants thus transformed are resistant to  
certain diseases, in particular to certain fungal or  
10 bacterial diseases. Consequently, the DNA sequence  
encoding thanatin may be integrated with the main  
objective of producing plants resistant to the said  
diseases, thanatin being effective against fungal  
diseases such as those caused by *Cercospora*, in  
15 particular *Cercospora beticola*, *Cladosporium*, in  
particular *Cladosporium herbarum*, *Fusarium*, in  
particular *Fusarium culmorum* or *Fusarium graminearum* or  
by *Phytophthora*, in particular *Phytophthora cinnamomi*.

The chimeric gene may also advantageously  
20 comprise at least one selectable marker, such as one or  
more herbicide tolerance genes.

The DNA sequence encoding thanatin may also  
be integrated as a selectable marker during the  
transformation of plants with other sequences encoding  
25 other peptides or proteins of interest such as, for  
example, herbicide tolerance genes.

Such herbicide tolerance genes are well known  
to a person skilled in the art and are in particular

described in patent applications EP 115 673,  
WO 87/04181, EP 337 899, WO 96/38567 or WO 97/04103.

Of course, the transformed cells and plants  
according to the invention may comprise, in addition to  
5 the sequence encoding thanatin, other heterologous  
sequences encoding other additional peptides capable of  
conferring on the plant resistance to other diseases of  
bacterial or fungal origin.

The other sequences may be integrated by  
10 means of the same vector comprising a chimeric gene,  
which comprises a first sequence encoding thanatin and  
at least one other sequence encoding another peptide or  
protein of interest.

They may also be integrated by means of  
15 another vector comprising at least the said other  
sequence, according to the customary techniques defined  
above.

The plants according to the invention may  
also be obtained by crossing parents, one carrying the  
20 gene according to the invention encoding thanatin, the  
other carrying a gene encoding at least one other  
peptide or protein of interest.

Among the sequences encoding other antifungal  
peptides, there may be mentioned that encoding  
25 drosomycin, which is described in patent application FR  
2,725,992 and by Fehlbauer et al. (1994), and in the  
unpublished patent application FR 97 09115 filed on  
24 July 1997, or that encoding androctonin described in

patent application FR 2,745,004 and in unpublished  
patent application FR 97 10362 filed on 20 August 1997.

The examples below make it possible to  
illustrate the invention, the preparation of the  
5 sequence encoding thanatin, of the chimeric gene, of  
the integration vector and of the transformed plants.  
Figures 1 to 5 in the annex describe the schematic  
structures of some plasmids prepared for the  
construction of chimeric genes. In these figures, the  
10 different restriction sites are marked in *italics*.

#### **Example 1: Construction of the chimeric genes**

All the techniques used below are standard  
laboratory techniques. The detailed protocols of these  
15 techniques are in particular described in Ausubel et  
al.

#### **pRPa-MD-P: Creation of a plasmid containing the signal peptide of the tobacco PR-1a gene**

20 The two complementary synthetic  
oligonucleotides Oligo 1 and Oligo 2 below are  
hybridized at 65°C for 5 minutes and then by slowly  
reducing the temperature to 30°C for 30'.

25 Oligo 1: 5' GCGTCGACGC GATGGGTTTC GTGCTTTTCT CTCAGCTTCC  
ATCTTTCCTT CTTGTGTCTA CTCTTCTTCT TTTCC 3'

Oligo 2: 5' TCGCCGGCAC GGCAAGAGTA AGAGATCACA AGGAAAAGAA  
GAAGAGTAGA CACAAGAAGG AAAGATGGAA GC 3'

After hybridization between Oligo 1 and Oligo  
5 2, the DNA remaining single-stranded serves as a  
template for the Klenow fragment of polymerase I of *E.*  
*coli* (under the standard conditions recommended by the  
manufacturer (New England Biolabs)) for the creation of  
the double-stranded oligonucleotide starting from the 3'  
10 end of each oligo. The double-stranded oligonucleotide  
obtained is then digested with the restriction enzymes  
*SacII* and *NaeI* and cloned into the plasmid pBS II SK(-)  
(Stratagene) digested with the same restriction  
enzymes. A clone is then obtained comprising the region  
15 encoding the signal peptide of the tobacco PR-1a gene  
(SEQ ID NO 3).

**pRPA-PS-PR1a-tha: Creation of a sequence encoding  
thanatin fused to the signal peptide PR-1a without a  
20 nontranscribed region in 3'**

The two synthetic oligonucleotides with  
complementary sequences Oligo 3 and Oligo 4 based on  
the operating conditions described for **pRPA-MD-P**.

25 Oligo 3: 5' GGTCCAAGA AGCCAGTGCC AATCATCTAC TGCAACAGGA CG 3'

[illegible][illegible][illegible][illegible]

contains the double CaMV 35S promoter isolated from the cauliflower mosaic virus (CaMV 2x35S promoter, Odell et al., 1985) which directs the expression of an RNA containing [lacuna] tobacco etch virus 5' untranslated  
 5 sequence (TEV 5' UTR; Carrington & Freed, 1990), the *E.coli*  $\beta$ -glucorinidase gene (GUS; Jefferson et al., 1987) followed by the polyadenylation site of the CaMV 35S RNA (CaMV polyA; Odell et al., 1985).

The plasmid pRTL-2 GUS is digested with the  
 10 restriction enzymes *NcoI* and *BamHI*, and the large DNA fragment is purified. The plasmid pRPA-PS-PR1a-than is digested with the restriction enzymes *NcoI* and *BamHI*, and the small DNA fragment containing the region encoding the fusion protein PR-1a-thanatin is purified.  
 15 The two DNA fragments purified are then ligated together in an expression cassette in plants which synthesizes a PR-1a-thanatin fusion protein. The schematic structure of this expression cassette is represented in Figure 2. "PR-1a-thanatin" represents  
 20 the coding region for the fusion protein PR-1a-thanatin of pRPA-RD-230. The thanatin is transported to the extracellular matrix of the plant by the action of the signal peptide PR-1a.

## 25 **pRPA-RD-195: Creation of a plasmid containing a modified multiple cloning site**

The plasmid pRPA-RD-195 is a plasmid derived from pUC-19 which contains a modified multiple cloning

site. The complementary synthetic oligonucleotides Oligo 5 and Oligo 6 below are hybridized and made double-stranded according to the procedure described for pRPA-MD-P.

5

Oligo 5:     5'     AGGGCCCCCT AGGGTTTAAA CGGCCAGTCA GGCCGAATTC  
                           GAGCTCGGTA CCCGGGGATC CTCTAGAGTC GACCTGCAGG  
                           CATGC 3'

10 Oligo 6:     5'     CCCTGAACCA GGCTCGAGGG CGCGCCTTAA TTAAAAGCTT  
                           GCATGCCTGC AGGTCGACTC TAGAGG 3'

The double-stranded oligonucleotide obtained is then ligated into pUC-19 which has been previously  
 15 digested with the restriction enzymes *EcoRI* and *HindIII* and made blunt ended using the Klenow fragment of DNA polymerase I of *E. coli*. A vector is obtained containing multiple cloning sites in order to facilitate the introduction of expression cassettes  
 20 into an *Agrobacterium tumefaciens* vector plasmid. The schematic structure of this multiple cloning site is represented in Figure 3.

**pRPA-RD-232: Introduction of the PR-1a-thanatin**  
 25 **expression cassette of pRPA-RD-229 into pRPA-RD-195**

The plasmid pRPA-RD-230 is digested with the restriction enzyme *HindIII*. The DNA fragment containing the PR-1a-thanatin expression cassette is purified. The



purified fragment is then ligated into pRPA-RP-195 which has previously been digested with the restriction enzyme *HindIII* and dephosphorylated with calf intestinal phosphatase.

5

**pRPA-RD-174**: Plasmid derived from pRPA-BL-150A (EP 0,508,909) containing the bromoxynil tolerance gene of pRPA-BL-237 (EP 0,508,909)

The bromoxynil tolerance gene is isolated  
 10 from pRPA-BL-237 by a PCR gene amplification. The fragment obtained is blunt-ended and is cloned into the *EcoRI* site of pRPA-BL-150A which has been made blunt-ended by the action of Klenow polymerase under standard conditions. An *Agrobacterium tumefaciens* vector is  
 15 obtained which contains the bromoxynil tolerance gene close to its right border, a kanamycin tolerance gene close to its left border and a multiple cloning site between these two genes.

The schematic structure of pRPA-RD-174 is  
 20 represented in Figure 4. In this figure, "nos" represents the *Agrobacterium tumefaciens* nopaline synthase polyadenylation site (Bevan et al., 1983), "NOS pro" represents the *Agrobacterium tumefaciens* nopaline synthase promoter (Bevan et al., 1983), "NPT  
 25 II" represents the neomycin phosphotransferase gene of the *E. coli* Tn5 transposon (Rothstein et al., 1981), "35S pro" represents the 35S promoter isolated from the cauliflower mosaic virus (Odell et al., 1985), "BRX"

5

10

15

20

25

A plasmid derived from pRPA-RD-174 is obtained comprising other restriction sites between the

bromoxynil tolerance gene and the selectable marker kanamycin gene.

The schematic structure of the plasmid pRPA-RD-184 is represented in Figure 5 where the terms  
 5 "nos", "NPT II", "NOS pro", "35S pro", "BRX gene", "RB" and "LB" have the same meaning as in Figure 4.

**pRPA-RD-235: Creation of an *Agrobacterium tumefaciens* vector containing the gene construct encoding thanatin**  
 10 **directed towards the extracellular matrix**

The plasmid pRPA-RD-232 is digested with the restriction enzymes *PmeI* and *AscI*, and the DNA fragment containing the gene for PR-la-thanatin is purified. The plasmid pRPA-RD-184 is digested with the same  
 15 restriction enzymes. The DNA fragment containing the PR-la-thanatin expression cassette is then ligated into pRPA-RD-184. An *Agrobacterium tumefaciens* vector is thus obtained containing the sequence encoding the fusion protein PR-la-thanatin which leads to the  
 20 expression of thanatin in the plant's extracellular matrix.

**Example 2: Herbicide tolerance of transformed tobaccos**

**2.1 - Transformation**

25 The vector pRPA-RD-235 is introduced into the *Agrobacterium tumefaciens* EHA101 strain (Hood et al., 1987) carrying the cosmid pTVK291 (Komari et al.,

1986). The transformation technique is based on the procedure of Horsch et al. (1985).

## **2.2 - Regeneration**

The regeneration of the tobacco PBD6 (origin SEITA France) from foliar explants is carried out on a Murashige and Skoog (MS) basal medium comprising 30 g/l of sucrose as well as 200 µg/ml of kanamycin. The foliar explants are removed from plants cultivated in a greenhouse or *in vitro* and regenerated according to the foliar disc technique (Horsch et al., 1985) in three successive stages: the first comprises the induction of shoots on a medium supplemented with 30 g/l of sucrose containing 0.05 mg/l of naphthylacetic acid (NAA) and 2 mg/l of benzylaminopurine (BAP) for 15 days. The shoots formed during this stage are then developed for 10 days by cultivating on an MS medium supplemented with 30 g/l of sucrose but containing no hormones. Next, the developed shoots are removed and they are cultivated on an MS rooting medium with half the content of salts, vitamins and sugar and containing no hormone. After about 15 days, the rooted shoots are transferred into the soil.

## **2.3 - Bromoxynil tolerance**

Twenty transformed plants were regenerated and transferred into a greenhouse for the pRPA-RD-235 construct. These plants were then treated in a greenhouse at the 5-leaf stage with an aqueous

suspension of Pardner corresponding to 0.2 kg of bromoxynil active material per hectare.

All the plants showing complete tolerance to bromoxynil are then used in various experiments which  
 5 show that the expression of thanatin by the transformed plants makes them resistant to fungal attacks.

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**CLAIMS**

1. Nucleic acid fragment, characterized in that it comprises a nucleic acid sequence encoding  
5 thanatin.

2. Nucleic acid fragment according to claim 1, characterized in that it is a DNA-type nucleotide sequence.

3. Nucleic acid fragment according to claim  
10 2, characterized in that the DNA-type nucleotide sequence comprises the DNA sequence described by the sequence identifier No. 1 (SEQ ID NO 1), a homologous sequence or a sequence complementary to the said sequence.

15 4. Nucleic acid fragment according to claim 3, characterized in that the DNA-type nucleotide sequence comprises the DNA sequence described by the sequence identifier No. 2 (SEQ ID NO 2), a homologous sequence or a sequence complementary to the said  
20 sequence.

5. Nucleic acid fragment according to one of claims 1 to 4, characterized in that it comprises a nucleic acid sequence fused in 5' and/or in 3' to the sequence encoding thanatin, so as to obtain a "protein-  
25 thanatin" fusion protein.

6. Nucleic acid fragment according to claim 5, characterized in that the protein is a signal peptide or a transit peptide.

7. Nucleic acid fragment according to claim 5 6, characterized in that the signal peptide is the signal peptide of the tobacco PR-1a gene.

8. Nucleic acid fragment according to claim 7, characterized in that it comprises the DNA sequence described by the sequence identifier No. 5 (SEQ ID NO 10 5), a homologous sequence or a sequence complementary to the said sequence.

9. Nucleic acid fragment according to claim 8, characterized in that it comprises the coding part of SEQ ID NO 5, corresponding to bases 12 to 164.

10. Fusion protein "protein-thanatin", characterized in that the protein is a signal peptide or a transit peptide.

11. Fusion protein according to claim 10, characterized in that the signal peptide is the signal 20 peptide of the tobacco PR-1a gene.

12. Fusion protein according to claim 11, characterized in that it is described by the sequence identifier No. 5 (SEQ ID NO 5).

13. Chimeric gene comprising a coding 25 sequence as well as heterologous regulatory elements at the 5' and 3' positions which can function in a host organism, in particular plants, characterized in that

the coding sequence comprises at least one DNA fragment encoding thanatin as defined in claims 1 to 9.

14. Chimeric gene according to claim 13, characterized in that the host organism is chosen from  
5 plant cells and plants.

15. Chimeric gene according to either of claims 13 and 14, characterized in that it also comprises a selectable marker.

16. Cloning or expression vector for the  
10 transformation of a host organism, characterized in that it comprises at least one replication origin and at least one chimeric gene as defined in claims 13 to 15.

17. Vector according to claim 16,  
15 characterized in that it is a virus used for the transformation of developed plants and containing, in addition, its own elements for replication and expression.

18. Vector according to claim 16,  
20 characterized in that it is a plasmid.

19. Transformed host organisms, characterized in that they contain an effective quantity of a chimeric gene according to claims 13 to 15.

20. Transformed host organism according to claim 19, characterized in that it consists of plant cells or plants.



21. Transformed host organism according to claim 20, characterized in that it is a plant containing transformed cells.

22. Host organism according to claim 21,  
5 characterized in that the plant is regenerated from transformed cells.

23. Transformed plant cell, characterized in that it contains a nucleic acid fragment according to claims 1 to 9, or a chimeric gene according to claims  
10 13 to 15.

24. Transformed plant resistant to diseases, characterized in that it comprises at least one transformed plant cell according to claim 23.

25. Transformed plant according to claim 24,  
15 characterized in that it is resistant to diseases caused by *Cercospora*, in particular *Cercospora beticola*, *Cladosporium*, in particular *Cladosporium herbarum*, *Fusarium*, in particular *Fusarium culmorum* or *Fusarium graminearum* or by *Phytophthora*, in particular  
20 *Phytophthora cinnamomi*.

26. Disease-resistant transformed plant, characterized in that it is derived from the cultivation and/or crossing of plants according to either of claims 24 and 25.

25 27. Seeds of transformed plants according to one of claims 24 to 26.

28. Method of transforming host organisms, in particular plant cells or plants, characterized in

that at least one nucleic acid fragment according to claims 1 to 9 or a chimeric gene according to one of claims 13 to 15 is inserted into the said host organism.

5                   29. Method of transforming plants to make them resistant to fungal or bacterial diseases, characterized in that at least one nucleic acid fragment according to claims 1 to 9 or a chimeric gene according to claims 13 to 15 is inserted into the  
10 plant.

                  30. Method according to either of claims 28 and 29, characterized in that the chimeric gene is inserted by means of a vector according to one of claims 16 to 18.

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

(111) NUMBER OF SEQUENCES: 13

## 5 (2) INFORMATION FOR SEQ ID NO: 1:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33 base pairs

(B) TYPE: nucleotide

(C) STRANDEDNESS: single

10 (D) TOPOLOGY: linear

(11) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION:1..33

## 15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

ATC ATC TAC TGC AAC AGG AGG ACT GGT AAG TGC

33

Ile Ile Tyr Cys Asn Arg Arg Thr Gly Lys Cys

1 5 10

## 20 (2) INFORMATION FOR SEQ ID NO: 2:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 63 base pairs

(B) TYPE: nucleotide

(C) STRANDEDNESS: single

25 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION:1..63

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

GGT TCC AAG AAG CCA GTG CCA ATC ATC TAC TGC AAC AGG AGG ACT GGT 48

Gly Ser Lys Lys Pro Val Pro Ile Ile Tyr Cys Asn Arg Arg Thr Gly

5 1 5 10 15

AAG TCG CAG AGG ATG

Lys Cys Gln Arg Met

20

10 (2) INFORMATION FOR SEQ ID NO: 3:

(1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 98 base pairs

(B) TYPE: nucleotide

(C) STRANDEDNESS: single

15 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION:1..63

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GGT TCC AAG AAG CCA GTG CCA ATC ATC TAC TGC AAC AGG AGG ACT GGT 48

Gly Ser Lys Lys Pro Val Pro Ile Ile Tyr Cys Asn Arg Arg Thr Gly

1 5 10 15

25 AAG TGC CAG AGG ATG TGAGCTCGGC GAGGCGAACG TGTCGACGGA TCCGG 98

Lys Cys Gln Arg Met

20

## (2) INFORMATION FOR SEQ ID NO: 4:

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 106 base pairs

(B) TYPE: nucleotide

5 (C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(11) MOLECULE TYPE: cDNA

(1x) FEATURE:

(A) NAME/KEY: CDS

10 (B) LOCATION:12..101

(x1) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

GCGTCGACGC C ATG GGT TTC GTG CTT TTC TCT CAG CTT CCA TCT TTC CTT 50  
 Met Gly Phe Val Leu Phe Ser Gln Leu Pro Ser Phe Leu  
 1 5 10  
 15 CTT GTG TCT ACT CTT CTT CTT TTC CTT GTG ATC TCT CAC TCT TGC CGT 98  
 Leu Val Ser Thr Leu Leu Leu Phe Leu Val Ile Ser His Ser Cys Arg  
 15 20 25

GCC GCGA

106

Ala

30

5 (2) INFORMATION FOR SEQ ID NO: 5:

(1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 197 base pairs

(B) TYPE: nucleotide

(C) STRANDEDNESS: single

10 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION:12..164

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

GCGTCGACGC C ATG GGT TTC GTG CTT TTC TCT CAG CTT CCA TCT TTC CTT 50

Met Gly Phe Val Leu Phe Ser Gln Leu Pro Ser Phe Leu

1 5 10

CTT GTG TCT AT CTT CTT CTT TTC CTT GTG ATC TCT CAC TCT TGC CGT 98

20 Leu Val Ser Thr Leu Leu Leu Phe Leu Val Ile Ser His Ser Cys Arg

15 20 25

GCC GGT TCC AAG AAG CCA GTG CCA ATC ATC TAC TGC AAC AGG AGG ACT 146

Ala Gly Ser Lys Lys Pro Val Pro Ile Ile Tyr Cys Asn Arg Arg Thr

30 35 40 45

25 GGT AAG TGC CAG AGG, ATG TGAGCTCGGC GAGGCGAACG TGTCGACGGA TCC 197

Gly Lys Cys Gln Arg Met

50

009101-12043000

## (2) INFORMATION FOR SEQ ID NO: 6:

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 75 base pairs

(B) TYPE: nucleotide

5 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: Other nucleic acid

(A) DESCRIPTION:/desc = "synthetic oligonucleotide 1"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

10 GCGTCGACGC GATGGGTTTC GTGCTTTTCT CTCAGCTTCC ATCTTTCCTT CTTGTGTCTA 60  
CTCTTCTTCT TTTCC

## (2) INFORMATION FOR SEQ ID NO: 7:

## (i) SEQUENCE CHARACTERISTICS:

15 (A) LENGTH: 72 base pairs

(B) TYPE: nucleotide

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: Other nucleic acid

20 (A) DESCRIPTION:/desc = "synthetic oligonucleotide 2"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

TCGCCGGCAC GGCAAGAGTA AGAGATCACA AGGAAAAGAA GAAGAGTAGA CACAAGAAGG 60  
AAAGATGGAA GC 72

## 25 (2) INFORMATION FOR SEQ ID NO: 8:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 44 base pairs

(B) TYPE: nucleotide

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other nucleic acid

(A) DESCRIPTION:/desc = "synthetic oligonucleotide 3"

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

GGTCCAAGA AGCCAGTGCC AATCATCTAC TGCAACAGGA CG

42

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

10 (A) LENGTH: 97 base pairs

(B) TYPE: nucleotide

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other nucleic acid

15 (A) DESCRIPTION:/desc = "synthetic oligonucleotide 4"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

CCGGATCCGT CGACACGTTT GCCTCGCCGA GTCACATCC TCTGGCACTT ACCAGTCCTC

60

CTGTTGCAGT AGATGATTGG CACTGGC

87

20

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 85 base pairs

(B) TYPE: nucleotide

25 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other nucleic acid

(A) DESCRIPTION:/desc = "synthetic oligonucleotide 5"



(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

AGGGCCCCCT AGGGTTTAAA CGGCCAGTCA GGCCGAATTC GAGCTCGGTA CCCGGGGATC 60  
CTCTAGAGTC GACCTGCAGG CATGC 85

5 (2) INFORMATION FOR SEQ ID NO: 110:

(1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 66 base pairs

(B) TYPE: nucleotide

(C) STRANDEDNESS: single

10 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other nucleic acid

(A) DESCRIPTION:/desc = "synthetic oligonucleotide 6"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

CCCTGAACCA GGCTCGAGGG CGCGCCTTAA TTAAAAGCTT GCATGCCTGC AGGTCGACTC TAGAGG 60

15

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 93 base pairs

(B) TYPE: nucleotide

20 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other nucleic acid

(A) DESCRIPTION:/desc = "synthetic oligonucleotide 7"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

25 CCGGCCAGTC AGGCCACACT TAATTAAGTT TAAACGCGGC CCCGGCGCGC CTAGGTGTGT 60  
GCTCGAGGGC CCAACCTCAG TACCTGGTTC AGG 93

(2) INFORMATION FOR SEQ ID NO: 13:

5

(B) TYPE: nucleotide

(C) TOPOLOGY: linear

(A) DESCRIPTION:/desc = "synthetic oligonucleotide 8"

(x1) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

10 CCGGCCTGAA CCAGGTACTG AGGTTGGGCC CTCGAGCACA CACCTAGGCG CGCCGGGGCC 60

GC GTTTAAAC TTAATTAAGT GTGGCCTGAC TGG 93

1/2

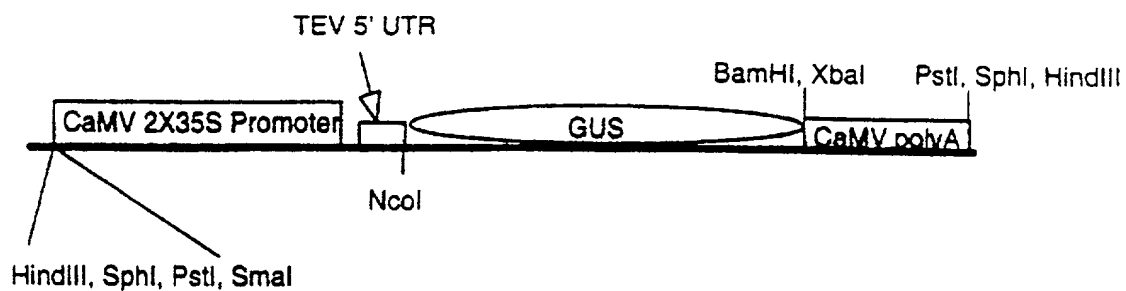


Fig. 1

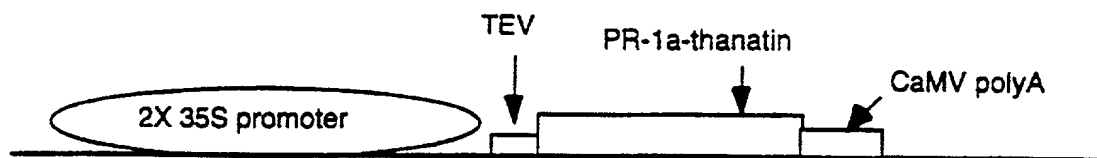


Fig. 2

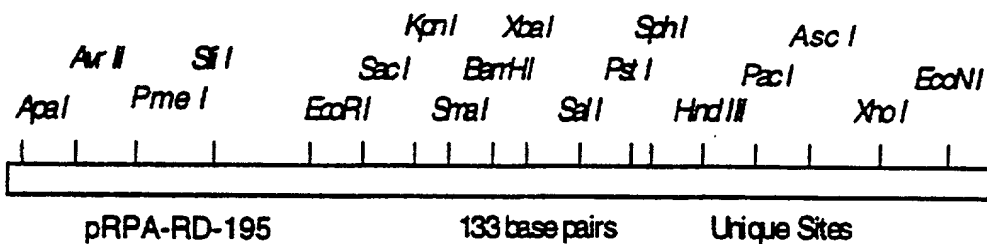


Fig. 3

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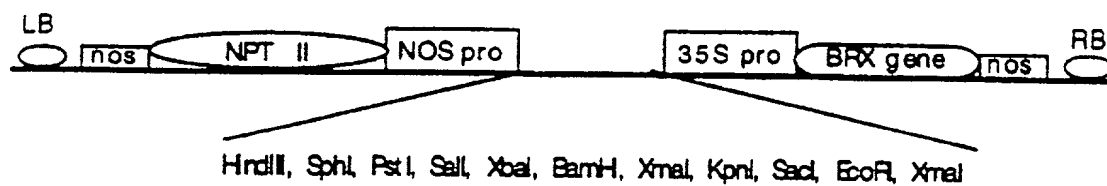


Fig. 4

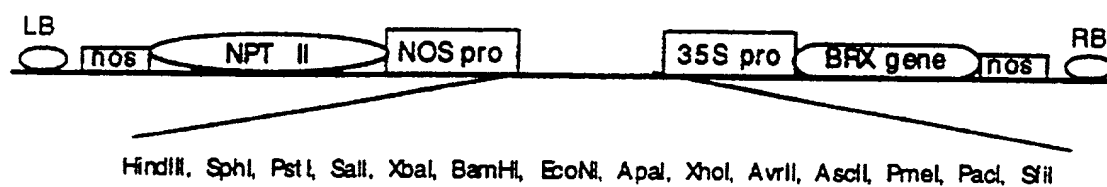


Fig. 5

**COMBINED DECLARATION  
AND POWER OF ATTORNEY**

**(Original, Design, National Stage of PCT, Divisional, Continuation or C-I-P Application)**

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name; I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

GENE CODING FOR THANATIN, VECTOR CONTAINING  
SAME AND RESULTING TRANSFORM DISEASE-RESISTANT PLANTS

This declaration is of the following type:

- ☐ original
- ☐ design
- ☒ national stage of PCT.
- ☐ divisional
- ☐ continuation
- ☐ continuation-in-part (C-I-P)

the specification of which: *(complete (a), (b), or (c))*

- (a) ☐ is attached hereto.
- (b) ☒ was filed on May 8, 2000 as Application Serial No. 09/554,024 and was amended on *(if applicable)*.
- (c) ☒ was described and claimed in PCT International Application No. PCT/FR98/02375 filed on November 6, 1998 and was amended on *(if applicable)*.

**Acknowledgement of Review of Papers and Duty of Candor**

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability of the subject matter claimed in this application in accordance with Title 37, Code of Federal Regulations § 1.56.

☐ In compliance with this duty there is attached an information disclosure statement. 37 CFR 1.98.

**Priority Claim**

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) of any foreign application(s) for patent or inventor's certificate or of any PCT International Application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT International Application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application on which priority is claimed

*(complete (d) or (e))*

- (d) ☐ no such applications have been filed.
- (e) ☒ such applications have been filed as follows:

PRIOR FOREIGN/PCT APPLICATION(S) FILED WITHIN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO SAID APPLICATION				
COUNTRY	APPLICATION NO.	DATE OF FILING (day, month, year)	DATE OF ISSUE (day, month, year)	PRIORITY CLAIMED UNDER 35 USC 119
France	97/14.263	November 7, 1997		<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO <input type="checkbox"/>
				<input type="checkbox"/> YES <input type="checkbox"/> NO <input type="checkbox"/>
				<input type="checkbox"/> YES <input type="checkbox"/> NO <input type="checkbox"/>
ALL FOREIGN APPLICATION[S], IF ANY, FILED MORE THAN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO SAID APPLICATION				
				<input type="checkbox"/> YES <input type="checkbox"/> NO <input type="checkbox"/>
				<input type="checkbox"/> YES <input type="checkbox"/> NO <input type="checkbox"/>
				<input type="checkbox"/> YES <input type="checkbox"/> NO <input type="checkbox"/>

### Claim for Benefit of Prior U.S. Provisional Application(s)

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below:

Provisional Application Number	Filing Date

### Claim for Benefit of Earlier U.S./PCT Application(s) under 35 U.S.C. 120

*(complete this part only if this is a divisional, continuation or C-I-P application)*

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior application(s) in the manner provided by the first paragraph of Title 35, United States Code § 112, I acknowledge the duty to disclose information as defined in Title 37, Code of Federal Regulations, § 1.56 which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
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(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
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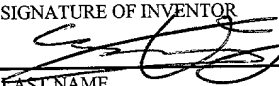

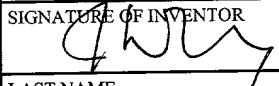
### Power of Attorney

As a named inventor, I hereby appoint Dana M. Raymond, Reg. No. 18,540; Frederick C. Carver, Reg. No. 17,021; Francis J. Hone, Reg. No. 18,662; Joseph D. Garon, Reg. No. 20,420; Arthur S. Tenser, Reg. No. 18,839; Ronald B. Hildreth, Reg. No. 19,498; Thomas R. Nesbitt, Jr., Reg. No. 22,075; Robert Neuner, Reg. No. 24,316; Richard G. Berkley, Reg. No. 25,465; Richard S. Clark, Reg. No. 26,154; Bradley B. Geist, Reg. No. 27,551; James J. Maune, Reg. No. 26,946; John D. Murnane, Reg. No. 29,836; Henry Tang, Reg. No. 29,705; Robert C. Scheinfeld, Reg. No. 31,300; John A. Fogarty, Jr., Reg. No. 22,348; Louis S. Sorell, Reg. No. 32,439; Rochelle K. Seide Reg. No. 32,300; Gary M. Butter, Reg. No. 33,841; Marta E. Delsignore, Reg. No. 32,689; and Lisa B. Kole, Reg. No. 35,225 of the firm of BAKER BOTTS L.L.P., with offices at 30 Rockefeller Plaza, New York, New York 10112, as attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith

SEND CORRESPONDENCE TO: BAKER BOTTS L.L.P. 30 ROCKEFELLER PLAZA, NEW YORK, N.Y. 10112 <u>CUSTOMER NUMBER: 21003</u>	DIRECT TELEPHONE CALLS TO: BAKER BOTTS L.L.P. (212) 705-5000
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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section

1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

FULL NAME OF SOLE OR FIRST INVENTOR	LAST NAME <b>FREYSSINET</b>	FIRST NAME <b>GEORGES</b>	MIDDLE NAME	
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DATE <b>03/29/2000</b>	SIGNATURE OF INVENTOR 			
FULL NAME OF SECOND JOINT INVENTOR, IF ANY	LAST NAME <b>DEROSE</b>	FIRST NAME <b>RICHARD</b>	MIDDLE NAME	
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DATE <b>09/14/00</b>	SIGNATURE OF INVENTOR 			
FULL NAME OF THIRD JOINT INVENTOR, IF ANY	LAST NAME <b>HOFFMANN</b>	FIRST NAME <b>JULES</b>	MIDDLE NAME	
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DATE <b>11.08.00</b>	SIGNATURE OF INVENTOR 			
FULL NAME OF FOURTH JOINT INVENTOR, IF ANY	LAST NAME	FIRST NAME	MIDDLE NAME	
RESIDENCE & CITIZENSHIP	CITY	STATE or FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP	
POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE or COUNTRY	ZIP CODE
DATE	SIGNATURE OF INVENTOR			
FULL NAME OF FIFTH JOINT INVENTOR, IF ANY	LAST NAME	FIRST NAME	MIDDLE NAME	
RESIDENCE & CITIZENSHIP	CITY	STATE or FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP	
POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE or COUNTRY	ZIP CODE
DATE	SIGNATURE OF INVENTOR			
FULL NAME OF SIXTH JOINT INVENTOR, IF ANY	LAST NAME	FIRST NAME	MIDDLE NAME	
RESIDENCE & CITIZENSHIP	CITY	STATE or FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP	
POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE or COUNTRY	ZIP CODE
DATE	SIGNATURE OF INVENTOR			